Hypobaric Hypoxia in Ascites Resistant and Susceptible Broiler Genetic Lines Influences Gut Morphology[1]

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ABSTRACT Genetic selection based on rapid growth rates, improved feed conversion, and increased body weights has led to a predisposition to ascites in broiler populations. Sire-family selection was applied to a commercial elite line to produce divergent lines of ascites-resistant (RES) and ascites-susceptible (SUS) broilers by the 8th generation. One objective of this research was to determine the effects of hypobaric hypoxia on gut morphology in these genetic lines. In two separate trials, pedigree broiler chickens were randomly assigned to cages in a hypobaric chamber (simulated 2,900 m above sea level) or a matching local altitude chamber (390 m above sea level). Ascites incidence was characterized by heart enlargement and fluid accumulation in the abdominal cavity. At the end of the study on d 42, all surviving birds were killed and evaluated for the presence of ascites and 2-cm sections from the duodenum and lower ileum were collected from 5 chickens per line, per altitude for each trial for morphometric analysis. At a high altitude, ascites incidence was lower in the RES line (20.9 and 3.7%) than in the SUS line (86.4 and 66.9%, Trials 1 and 2, respectively). No ascites was observed at a local altitude. Under hypoxic conditions, duodenum villus surface area was higher ($P < 0.05$) in the RES line (181.3 ± 16.8 and 219 ± 10.9 μm) compared with the SUS line (130.1 ± 10.5 and 134.3 ± 9.3 μm; Trials 1 and 2, respectively). No differences in ileum villus morphology were observed for any of the parameters measured. The reduced surface area in the duodenum of birds selected for ascites susceptibility suggests reduced enteric function and may provide clues as to why these birds have increased incidence of ascites.

(*Key words*: ascites, gut morphology, altitude, genetic lines, hypoxia)

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INTRODUCTION

The modern broiler has been intensely selected for improved growth rates and increased feed conversion (Pakdel et al., 2002). Broilers from the 1950s required 14 wk to reach market body weight, whereas birds today are ready for market at 6 wk of age with a body weight of 2.6 kg (Havenstein et al., 1994). Unfortunately, lung capacity does not always meet the oxygen needed for the rapid growth observed in fast growing broilers, which can result in hypoxemia and ascites (Julian, 2000). Ascites is a metabolic disease characterized by hypertrophy of the right ventricle, a flaccid heart, and an accumulation of fluid in the abdominal cavity (Riddell, 1991; Chapman and Wideman, 2001; Balog, 2003). Ascites usually occurs in broilers reared at high altitudes where the partial pressure of oxygen is reduced (Owen et al., 1990; Wideman et al., 2003). It is estimated that 5% of broilers and 20% of roaster birds die of ascites (Swire, 1980; Julian et al., 1986); considering that an estimated 40 billion broilers are produced annually around the world, it is evident that the economic losses due to ascites are significant.

Genetic selection may provide producers with a tool for preventing ascites, while increasing bird performance. Lubritz and colleagues (1995) report moderate to high heritability for ascites that range from 0.11 to 0.44. A positive genetic correlation between ascites and right ventricle-to-total ventricle ratio (RV/TV) also was reported, suggesting that selection for a decreased RV/TV ratio would simultaneously reduce the incidence of ascites (Lubritz et al., 1995). Moghadam and coworkers (2001) estimated genetic parameters for ascites syndrome in the White Rock and Cornish breeds and found heritabilities that were consistent with those of Lubritz and associates (1995). Using a hypobaric chamber, we have developed

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Abbreviation Key: GIT = gastrointestinal tract, RES = resistant, RV/TV = right ventricle-to-total ventricle ratio, SUS = susceptible.

lines that are resistant (RES) or susceptible (SUS) to ascites that are in the 8th generation of selection. We reported an increased RV/TV and heavier organ weights in the SUS line compared with the RES, which are risk factors for ascites (Anthony et al., 2001; Balog et al., 2003). The differences observed in organ weights between the RES and SUS lines led us to look at villus development in the gastrointestinal tract (GIT) to evaluate for differences in gut morphology. The GIT is a metabolically active system that has considerable nutrient and oxygen requirements. Although the total oxygen demand of the gut is not known for chickens, the pig GIT uses 25% of total oxygen consumption and represents only 5% of its total body weight (Yen et al., 1989). The high oxygen demand of the GIT on the heart and lungs may explain why feed restriction can reduce ascites incidence in broilers; however, decreasing feed consumption can also decrease productivity (Balog et al., 2000). We hypothesize that the enteric tract of birds selected to be resistant to ascites are healthier and more efficient than those of birds that are susceptible to ascites. The objective of this study was to evaluate the gastrointestinal morphology of birds from divergent lines to investigate this hypothesis.

MATERIALS AND METHODS

Experimental Animals

Ascites RES and SUS lines were derived from a commercial elite line that had undergone a single generation of relaxed selection prior to initiation of ascites selection. Sire family selection was applied for the divergent lines (Balog et al., 2003). Each generation, 2 hatches of progeny derived from 24 sires mated to 3 hens per sire were reared for 6 wk under simulated high-altitude conditions in a hypobaric chamber or at a local altitude (Balog et al., 2003). Mortality data generated from the altitude treatments were used to select the most SUS and RES sire families to reproduce the lines at a local altitude. For each line, male breeders were selected from the top 6 sire families, and hens were selected from no more than the top 10 sire families. After 8 generations of selection, under hypobaric conditions, the ascites RES line exhibited 26.0% mortality and the SUS line exhibited 98.6% mortality (Pavlidis, 2003).

Housing and Diet

Day-of-hatch chicks were placed in stainless steel battery units housed in environmentally matched chambers at a local altitude or one that simulated a high-altitude, low-oxygen environment by creating a partial vacuum (Balog et al., 2000). Both chambers measured 2.4 × 3.7 × 2.4 m and were matched in terms of temperature and ventilation. Daily management tasks and weekly weighing were conducted under the partial vacuum through the use of an airlock that allowed for pressure equilibration (Balog et al., 2000). Chicks were fed ad libitum a corn-soybean broiler diet formulated without antibiotics or coccidiostats that met or exceeded levels of critical nutrients recommended by the NRC (1994).

Experimental Design

Two replicates were conducted to evaluate gut morphology in ascites RES and SUS broiler genetic lines. In each experiment, the lines were evaluated at a local altitude of 390 m above sea level and at a simulated high altitude of 2,900 m above sea level. Four hundred eighty birds were distributed in 40 pens of 12 birds per pen (20 pens per altitude for each trial) to evaluate ascites. A large sample size was used due to high ascites mortality in the SUS line. Birds were checked twice daily to record mortality and to determine if mortalities were due to ascites. All mortalities were necropsied to determine cause of death. Ascites incidence was characterized by heart enlargement and fluid accumulation in the abdominal cavity, as previously described by Balog and coworkers (2003). At the end of the study, on d 42, all surviving birds were euthanized and evaluated for the presence of ascites. Total ascites incidence was determined by combining ascites mortality data throughout the study and the ascites incidence present on the last day of the experiment. Gastrointestinal samples were randomly collected from 5 ascites-free birds per line, per altitude, per trial on d 42 for morphometric analysis.

Morphometric Analysis of the Gut

The gastrointestinal morphometric variables evaluated were villus height, villus surface area, lamina propria thickness, and villus crypt depth from the duodenum and ileum. On d 42, a 2-cm segment of the midpoint of the duodenum and the distal end of the lower ileum were dissected and fixed in 10% buffered formalin for 72 h. Each segment was embedded in paraffin. A 2-μm section of each sample was placed onto a glass slide and stained with hematoxylin & eosin for examination with a light microscope (Sakamoto et al., 2000). Morphological parameters were measured with the Image Pro Plus v. 4.5 software package.[3] Each section represented a single chicken. Twenty replicate measurements for each variable studied were taken from each chicken. These 20 measurements were then averaged to generate a mean value for each variable for an individual chicken. The villus height was measured from the top of the villus to the top of the lamina propria. Surface area was calculated using the formula = $(2\pi) \times (VW/2) \times (VL)$ in which VW = villus width and VL = villus length (Sakamoto et al., 2000). The lamina propria thickness was measured in the space between the base of the villus and the top of the muscularis mucosa. Crypt depth was measured from the base upward to the region of transition between the crypt and villus (Aptekmann et al., 2001).

[3]MediaCybernetic, Silver Spring, MD.

TABLE 1. Effect of hypoxia and genetic selection on the duodenum morphology, mortality and ascites incidence of broilers

Variables	Local altitude	High altitude
Villus surface area (μm^2)*		
Trial 1		
Resistant	220.8 ± 30.7^{a,x}	181.3 ± 16.8^{a,x}
Susceptible	248.8 ± 20.1^{a,x}	130.1 ± 10.5^{b,y}
Trial 2		
Resistant	264.6 ± 35.4^{a,x}	219.6 ± 10.9^{a,x}
Susceptible	171.5 ± 12.5^{b,x}	134.3 ± 9.3^{b,y}
Crypt depth, μm[1]		
Trial 1		
Resistant	8.8 ± 0.5^{a,x}	6.8 ± 0.3^{a,y}
Susceptible	5.8 ± 0.2^{b,x}	6.5 ± 0.3^{a,x}
Trial 2		
Resistant	9.3 ± 0.6^{a,x}	8.9 ± 0.3^{a,x}
Susceptible	6.2 ± 0.3^{b,x}	7.0 ± 0.3^{b,x}
Villus height, μm[1]		
Trial 1		
Resistant	40.2 ± 5.2^{a,x}	34.5 ± 3.3^{a,x}
Susceptible	39.3 ± 0.1^{a,x}	31.9 ± 1.9^{a,y}
Trial 2		
Resistant	44.8 ± 1.9^{a,x}	42.6 ± 3.3^{a,x}
Susceptible	34.7 ± 2.1^{b,x}	30.0 ± 1.9^{b,x}
Lamina propria thickness, μm[1]		
Trial 1		
Resistant	5.1 ± 0.3^{a,x}	5.5 ± 0.1^{a,x}
Susceptible	5.3 ± 0.2^{a,x}	5.0 ± 0.1^{b,x}
Trial 2		
Resistant	6.5 ± 0.7^{a,x}	5.0 ± 0.1^{a,y}
Susceptible	4.6 ± 0.4^{bx}	4.9 ± 0.1^{a,x}
Ascites incidence, %		
Trial 1		
Resistant	0	20.9^{a}
Susceptible	0	86.4^{b}
Trial 2		
Resistant	0	3.7^{a}
Susceptible	0	66.9^{b}
Total mortality, %		
Trial 1		
Resistant	3.8^{a}	27.3^{a}
Susceptible	3.6^{a}	92.7^{b}
Trial 2		
Resistant	3.9^{a}	6.8^{a}
Susceptible	3.9^{a}	85.5^{b}

[1]Mean ± SEM representing 5 birds per group and the average of 20 measurements per parameter, per bird.

^{a,b}Significant at $P < 0.05$ between treatments within altitudes (columns).

^{x,y}Significant at $P < 0.05$ within treatments between altitudes (rows).

Statistical Analysis

All percentage data were subjected to arc sine transformation. The experimental design employed a 2 × 2 factorial arrangement of treatments. Gut morphology data were subjected to ANOVA using SAS (SAS Institute, 1988). Mean separation was accomplished using Duncan's multiple range test (Duncan, 1955). A probability value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

At a high altitude, ascites incidence was lower in the RES line (20.9 and 3.7%) than the SUS line (86.4 and 66.9%, Trials 1 and 2, respectively, Table 1). Ascites was not observed at local altitude for either of these genetic lines. These results were similar to earlier generations where ascites incidence was higher in the SUS compared with the RES line (Anthony et al., 2001). Overall mortality at local altitude was less than 4% for both lines over the course of the study. In the hypobaric chamber, total mortality was 27.3 and 6.8% for RES birds and 92.7 and 85.5% for SUS birds, Trials 1 and 2 and respectively (Table 1).

The duodenum villus surface area was consistently lower ($P < 0.05$) in SUS birds compared with RES birds reared in the hypobaric chamber (Table 1). In addition, the duodenum villus surface area of SUS birds was lower ($P < 0.05$) in the hypobaric chamber compared with birds reared at local altitude (Table 1). Although only observed in one trial (Trial 2), duodenum villus surface area of SUS birds reared at a local altitude was lower than that of RES birds. Duodenal surface area appears to be influenced by genetic differences in the lines as well as the stress of the high-altitude simulation. When subjected to the hypobaric chamber, the SUS birds had a lower surface area compared with the RES birds in the same conditions. Interestingly the SUS birds in the simulated high-altitude condition had a reduced surface area compared with local altitude, whereas the surface area in the RES birds was not different due to altitude.

Duodenum crypt depth was significantly deeper in the RES line compared with the SUS line at local altitude. Similar results were observed at high altitude in Trial 2, where the RES line duodenum crypt depth was deeper when compared with the SUS line. In addition, crypt depth was shallower in RES birds ($P < 0.05$) in the hypoxic environment compared with birds reared at local altitude in Trial 1 (Table 1). The number and maturity of epithelial cells in the crypt and the villus determine the activity of the striated brush border (Brown, 1962). The proliferation of these regenerative cells is consistent with increases in crypt depth and villus height (Jervis and Levin, 1966). An increased crypt depth in the RES line may explain the larger villus surface area and villus height observed in this genetic line due to a more efficient turnover of regenerative cells along the crypts of the villus.

Duodenum villus height and lamina propria thickness were not as consistent between environments and genetic lines as the villus surface area or crypt depth measurements. Duodenum villus height was greater ($P < 0.05$) in the RES line compared with the SUS line in Trial 2 at both altitudes, but no differences were observed within altitudes in Trial 1. However, duodenum villus height was significantly reduced in the SUS line reared at high altitude when compared with the SUS line reared at a local altitude in Trial 1 (Table 1). The duodenum lamina propria was thicker ($P < 0.05$) in the RES line compared with the SUS line when reared at a high altitude, in Trial 1 (Table 1). At a local elevation in Trial 2, the duodenum villus lamina propria was 30% thicker in the RES line compared to the SUS line (Table 1). In addition, the duodenum villus lamina propria of RES birds were lower ($P < 0.05$) in the hypoxic environment compared with birds reared at local altitude (Table 1). No significant differences were observed for any of the variables evaluated

in the ileum when comparing altitude or genetic line treatments.

In this study, hypoxic conditions significantly reduced overall gut architecture in the SUS line. The gastrointestinal tract requires a large amount of oxygen (Yen et al., 1989) and hypoxia has been previously demonstrated to inhibit gut development in commercial broilers (Solis de los Santos et al., 2005). The SUS chicken is a larger animal than the RES chicken, which may predispose it to having increased ascites incidence because of the increased oxygen requirement (Pavlidis, 2003). These data suggest that in addition to increased ascites incidence, the higher oxygen demand required for the SUS line may decrease gut efficiency. The reduction in nutrient absorption may have also resulted in the increased relative gut weights previously observed in the SUS lines to compensate for this decreased gut efficiency (Pavlidis, 2003).

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